REMARKS

Claims 16-25 are currently pending in the above-identified application.

Claim 26 has been withdrawn from consideration as being directed to a non-elected invention.

Typographical errors in the specification have been corrected. Support for replacing cysteine γ synthase with cysteine synthase can be found in the drawings as originally filed and in information provided for the NCBI GI numbers in the NCBI database. For example,a) in the Brief Description of the Figures (Figure 1, page 5 at line 5, the description as originally filed indicated that CS was cysteine synthases; and b) the NCBI GI No. for *C. lanatus*, 540497, indicates the activity as being cysteine synthase in the NCBI database. Copies of this information for *C. lanatus*, *S. oleracea* and *S. tuberosum* are attached hereto.

Appropriate corrections have been made elsewhere in the specification as shown above so that the specification is consistent. Thus, it is believed that no new matter has been added.

Appropriate corrections have been made to the Brief Description of the Drawings as requested on page 3 of the Office Action. Corrections have also been made elsewhere in the specification for purposes of consistency. Thus, it is believed that no new matter has been added.

Claims 16, 17 and 20-25 were rejected under 35 USC §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Submitted herewith are copies of the following which set forth structural features common to cysteine synthase, also known as, O-acetylserine(thiol)lyase:

> Saito et al. (*FEBS Lett.* 328(1-2):111-114 (1993)) ("Saito")
> Determination of a functional lysine residue of a plant cysteine synthase by site-directed mutagenesis, and the molecular evolutional implications
>
> Barroso et al. (*FEBS Lett.* 363(1-2):1-5 (1995)) ("Barroso")
> A new member of the cytosolic *O*-acetylserine(thiol)lyase gene family in *Arabidopsis thaliana*
>
> Nakamura et al. (*Gene* 229:155-161 (1999)) ("Nakamura")
> Four rice genes encoding cysteine synthase: isolation and differential responses to sulfur, nitrogen and light

Saito was previously cited and Barroso and Nakamura are cited in a Supplemental IDS filed simultaneously herewith. Although Nakamura has a publication date after the earliest filing date of the instant application, it is respectfully submitted that Nakamura represents the state of knowledge of one of ordinary skill in the art, as of the earliest filing date of the instant application, at least with respect to

the conserved N-terminal motif and the lysine residue that is necessary for the catalytic activity of cysteine synthase.

Saito shows that Lys-49 is a functional residue needed for catalytic activity of cysteine synthase. Parenthetically it is noted that Saito et al., 1993b is identical to the Saito cited previously by Applicants.

Appendix A, attached hereto, is a Clustal V alignment of the amino acid sequence set forth in SEQ ID NO:31 of the instant application and the five cysteine synthase proteins found in either the instant specification, Barroso or the National Center for Biotechnology Information (NCBI) website:

1. SEQ ID NO:32 of the instant specification: cysteine synthase (*Citrullus lanatus*) (GI 540497)
2. cysteine synthase (*Arabidopsis thaliana*) (GI 804950) (disclosed in Barroso)
3. SEQ ID NO:66 of the instant specification: cysteine synthase (*Solanum tuberosum*) (GI 11131628)
4. cysteine synthase (*Spinacia oleracea*) (GI 12644209) (NCBI website – On February 1, 2001, this sequence version replaced GI 416869)
5. SEQ ID NO:65 of the instant specification: cysteine synthase (*Spinacia oleracea*) (GI 416869) (Appendix B, attached hereto, shows that #4 (GI 12644209) and #5 (GI 416869) are 99.7% identical.)

Appendix B, submitted herewith, is a chart setting forth a comparison of the percent identity (and percent divergence in the lower half triangle), among the five cysteine synthase proteins described above and the sequence set forth in SEQ ID NO:31.

The abstract of Nakamura states that "The predicted amino acid sequences contain the conserved PXXSVKDR region characteristic of cysteine synthase, which includes the lysine residue that binds the cofactor, pyridoxal 5'-phosphate."

Furthermore, page 156, column two of Nakamura states "This is confirmed by the presence of a Lys residue, which binds the pyridoxal 5'-phosphate cofactor (Saito et al., 1993b) and the neighboring residues (PXXSVKDR), which are well conserved among plant CSs (Fig. 1)."

The residues boxed in Appendix A shows that the sequence set forth in SEQ ID NO:31 of the instant application does indeed contain this essential conserved region and the lysine residue involved in the pyridoxal 5'-phosphate binding site is present and marked with an asterisk.

Given the above, it is respectfully submitted that claimed cysteine synthase polypeptide does possess structural features associated with cysteine synthases, for

example, the conserved PXXSVKDR region and lysine residue that binds the cofactor.

Clearly, one of ordinary skill would readily appreciate that amino acid substitutions are possible so that a polypeptide sequence having 90% sequence identity to SEQ ID NO:31 can be made and still retain cysteine synthase activity useful in cysteine biosynthesis.

In view of the foregoing discussion, withdrawal of the written description rejection under 35 USC § 112, first paragraph, is respectfully requested.

Claims 16-25 were rejected under 35 USC § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

It is respectfully submitted that the above-remarks pertaining to the written description rejection are equally apposite with respect to this ground of rejection.

Furthermore, submitted herewith are additional articles that appear to be representative of the knowledge of one of ordinary skill in the art:

Chronis et al. (*Crop Sci.* 43:1819-1827 (2003)) ("Chronis")
Sulfur Assimilation in Soybean: Molecular Cloning and Characterization of *O*-Acetylserine (Thiol) Lyase (Cysteine Synthase)

Sirko et al. (*J. Exp. Bot.* 55(404):1881-1888 (2004)) ("Sirko")
Overproduction of SAT and/or OASTL in transgenic plants: a survey of effect

Youssefian et al. (*Plant Physiol.* 126:1001-1011 (2001)) ("Youssefian")
Increased Cysteine Biosynthesis Capacity of Transgenic Tobacco Overexpressing an *O*-Acetylserine(thiol) Lyase Modified Plant Responses to Oxidative Stress

Noji et al. (*Plant Physiol.* 126:973-980 (2001)) ("Noji")
Cysteine Synthase Overexpression in Tobacco Confers Tolerance to Sulfur-Containing Environmental Pollutants

Chronis, Sirko, Youssefian and Noji are cited in a Supplemental IDS filed simultaneously herewith.

Although Sirko has a publication date after the filing date of the instant application, it is respectfully submitted that Sirko represents the state of knowledge of one of ordinary skill in the art, as of the filing date of the instant application, at least with respect to most of the information found in Table 2 (page 1885).
Table 2 of Sirko is a summary of the published works containing information concerning cysteine synthase (OASTL) overproduction in transgenic plants.

Appendix C, attached hereto, is a Clustal V alignment of the sequence set forth in SEQ ID NO:31 of the instant application and the soybean cysteine synthase protein disclosed in Chronis (*Glycine max* cysteine synthase mRNA; GI 18252505;

AF452451). The sequence set forth in SEQ ID NO:31 is 99.7 percent identical to the soybean cysteine synthase disclosed in Chronis.

It is stated on page 1825 of Chronis that soybean "OAS-TL contains the conserved PXXSVKDR motif that is characteristic of cysteine synthase. The lysine residue in this conserved motif has been shown to bind the co-factor pyridoxal 5'-phosphate. . . . "

Chronis confirmed the activity of the isolated cDNA by functional complementation of an *Escherichia coli* cysteine auxotrophic mutant. Given the high sequence identity between the sequence disclosed in Chronis and sequence set forth in SEQ ID NO:31, given that both of these sequences possess the above-identified conserved motif and lysine residue in the conserved motif, it appears that the sequence set forth in SEQ ID NO:31 is, indeed, a cysteine synthase.

Reliance on Wirtz (cited on page 6 of the Office Action) appears to be misplaced. Wirtz was cited for the proposition that since SAT is subject to feedback inhibition, then, transforming a plant with a nucleic acid encoding a CS will not overcome the feedback inhibition of SAT. This proposition appears to be contrary to work be done by researchers exploring overproduction of OASTL in transgenic plants.

Attention is also kindly invited to Sirko which presents a review concerning overproduction of SAT and/or OASTL in transgenic plants – a survey of effects. The Sirko abstract states, *inter alia*, that during "the last decade transgenic plants overproducing SAT, OASTL or both enzymes simultaneously were obtained independently by several research groups. These manipulations led not only to the elevated levels of the respective products, namely OAS and cysteine, but also to increased amounts of glutathione and changes in the levels of other metabolites and enzymatic activities. . . ."

The work of Youssefian and Noji (cited in Table 2 of Sirko relating to overproduction of OASTL) are just two examples of a plant being transformed with a cysteine synthase resulting in an increased concentration of cysteine.

Youssefian et al. described increased cysteine biosynthesis capacity of transgenic tobacco overexpressing an O-acetylserine(thiol) lyase as modifying plant responses to oxidative stress. It is stated on page 1006, column two, "The primary effect of the enhanced cytosolic OASTL activities of the *cys1* transgenic plants was to elevate CYS contents to over 2-fold those of control plants in the absence of any imposed stress."

Figure 5A of Noji (found on page 977) shows an increased concentration of cysteine in transgenic tobacco plants that were enhanced with cysteine synthase activities in the cytosol and in the chloroplasts compared to negative control plants. It

is stated on page 978, column 1 of Noji that "Our observations suggest that the transgenic plants in which the ability of Cys synthesis has been enhanced in the cytosol and in the chloroplasts by overexpressing CSase may be applied to product the transgenic plants resistant to oxidative stress caused by the photochemical oxidant such as ozone."

In view of the above discussion, it is respectfully submitted that one of ordinary skill in the art would be able to practice the claimed invention without engaging in undue experimentation and that feed-back inhibition of SAT would not be an impediment to increasing cysteine levels in a plant. Saito showed the active lysine residue for the catalytic action of cysteine synthase from spinach by site-directed mutagenesis at twelve different lysine resides in 1993. The mutants were readily transformed into an *Escherichia coli* cysteine auxotroph and assayed for activity.

Cysteine synthase activity may be determined using routine tests well known to those skilled in the art such as those disclosed in the instant specification on page 45, lines 14-16.

Accordingly, withdrawal of the enablement rejection of the claims under 35 USC §112, first paragraph, is respectfully requested.

A petition for a three (3) month extension of time accompanies this response along with a supplemental information disclosure statement and copies of the references cited therein.

It is respectfully submitted that the application is now in form for allowance which allowance is respectfully requested.

Please charge any fees or credit any overpayments of fees which are required in connection with the filing of this Response including, but not limited to, the Three Month Extension of Time to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Respectfully submitted,

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